

CLAIMS

~~sub~~ 1. A method of improving expression levels of one or more proteins in a transgenic plant comprising inserting into the genome of said plant a DNA sequence comprising a promoter region operably linked to two or more protein encoding regions and a 3'-terminator region wherein said protein encoding regions are separated from each other by a DNA sequence coding for a linker propeptide, said propeptide providing a cleavage site whereby the expressed polyprotein is post-translationally processed into the component protein molecules.

5 2. A method according to claim 1 wherein said promoter region is operably linked to a signal sequence, said signal sequence being operably linked to the said two or more protein encoding regions and a 3'-terminator region.

10 3. ~~sub~~ A method for the expression of multiple proteins in a transgenic plant comprising inserting into the genome of said plant a DNA sequence comprising a promoter region operably linked to a signal sequence said signal sequence being operably linked to two or more protein encoding regions and a 3'-terminator region wherein said protein encoding regions are separated from each other by a DNA sequence coding for a linker propeptide said propeptide providing a cleavage site whereby the expressed polyprotein is post-translationally processed into the component protein molecules.

15 20 4. A method according to any of the preceding claims wherein at least 40% of the sequence of said linker propeptide consists of stretches of either two to five consecutive hydrophobic residues selected from alanine, valine, isoleucine, methionine, leucine, phenylalanine, tryptophan and tyrosine or stretches of two to five hydrophilic residues selected from aspartic acid, glutamic acid, lysine, arginine, histidine, serine, threonine, glutamine and asparagine.

25 5. A method according to any of the preceding claims wherein said linker propeptide has within 7 residues of its N- or C-terminal cleavage site a sequence with two to five

consecutive acidic residues, two to five basic residues or two to five consecutive intermixed acidic and basic residues.

6. A method according to any of the preceding claims wherein the DNA sequence encoding said linker propeptide encodes a propeptide isolatable from a plant protein, or a virus or a variant thereof or a fragment of either of these which provides a cleavage site whereby the expressed polyprotein is post-translationally processed into the component protein molecules.

10 7. A method according to any of the preceding claims wherein the DNA sequence encoding said linker propeptide encodes a propeptide isolatable from a plant protein or a fragment thereof.

15 8. A method according to claim 6 or claim 7 wherein the DNA sequence encoding said linker propeptide encodes a chimeric propeptide comprising a propeptide isolatable from one or more plant proteins and/or a virus, or a variant thereof or a fragment of either of these.

20 9. A method according to any one of claim 7 or claim 8 wherein the plant protein is a precursor of a plant defensin, or a hevein-type antimicrobial protein .

10. A method according to claim 9 wherein the plant protein is an antimicrobial protein derived from the genus Impatiens.

25 11. A method according to claim 10 wherein the propeptide comprises SEQ ID NO. 3, 29, 21, 22, 23 or 24.

12. A method according to claim 8 wherein the propeptide comprises a C-terminal propeptide from Dm-AMP1 or Ac-AMP2 or a fragment thereof, or a variant of any of these.

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13. A method according to claim 12 wherein the propeptide comprises SEQ ID NO. 4, 6, 7, 25, 26 or 27.

14. A method according to any one of the preceding claims wherein the propeptide is a 5 chimeric propeptide.

15. A method according to any one of claim 13 wherein the chimeric propeptide comprises a virus propeptide or a fragment thereof, and a propeptide isolated from a plant protein or a fragment thereof.

10 16. A method according to claim 15 wherein the virus is a picornovirus.

17. A method according to claim 15 or 16 wherein the chimeric propeptide comprises SEQ ID NO 28 as the virus propeptide sequence.

15 18. A method according to any of the preceding claims wherein the linker propeptide has a protease processing site engineered at either or both ends thereof.

19. A method according to claim 18 wherein the protease processing site is a subtilisin - 20 like protease processing site.

20 21. A method according to claim 2 or 3 wherein the signal sequence is derived from a plant defensin gene.

25 22. A method according to any of the preceding claims wherein one or more of the multiple proteins is a defense protein.

23 23. Use of a propeptide cleavable in the secretory pathway of a plant linker for a polyprotein precursor synthesized in a transgenic plant.

23. Use of a propeptide according to claim 22 wherein the propeptide is derived from a plant protein or from a virus.

24. Use of a propeptide according to claim 22 or claim 23 wherein the propeptide is derived from a plant protein and the protein is a precursor of a plant defensin, or a hevein-type antimicrobial protein or is isolatable from the genus *Impatiens*.

25. Use of a propeptide as a cleavable linker in polyprotein precursors synthesized via the secretory pathway in transgenic plants wherein said propeptide linker is as defined in claim 4 or claim 5.

26. Use of a propeptide sequence rich in the small amino acids A, V, S and T and containing dipeptidic sequences consisting of either two acidic residues, two basic residues or one acidic and one basic residue as a cleavable linker sequence wherein said sequence is isolatable from a plant defensin or a hevein-type antimicrobial peptide.

27. A DNA construct comprising a DNA sequence comprising a promoter region operably linked to a plant derived signal sequence said signal sequence being operably linked to two or more protein encoding regions and a 3' terminator-region wherein said protein encoding regions are separated from each other by a DNA sequence coding for a linker propeptide said propeptide providing a post-translational cleavage site.

28. A DNA construct comprising a DNA sequence comprising a promoter region operably linked to two or more protein encoding regions and a 3' terminator-region wherein said protein encoding regions are separated from each other by a DNA sequence coding for a linker propeptide encoding a C-terminal propeptide from the Dm-AMP gene or from the Ac-AMP gene, said propeptide providing a post-translational cleavage site

29. A DNA construct according to claim 27 or claim 28 wherein the DNA sequence encoding the linker propeptide additionally comprises one or more protease recognition sites at either or both ends thereof.

5 30. A vector comprising a DNA construct according to any of claims 19 to 21.

31. A transgenic plant transformed with a DNA construct or a vector according to any one of claims 27 to 30.

32. Use of a DNA construct comprising a DNA sequence comprising a promoter region operably linked to two or more protein encoding regions and a 3' terminator region wherein said promoter encoding region are separated from each other by a DNA sequence coding for a linker propeptide, said propeptide providing a post-translational cleavage site for increasing protein expression levels in a transgenic plant, or a vector comprising said construct, for increasing protein expression levels in a transgenic plant.

20 33. A nucleic acid which encodes a peptide of SEQ ID NO 4, 6, 7, 29, 21, 22, 23, 24, 25, 26, 27, 28 or the linker peptide shown in Figure 34 or a variant of any of these.

34. A nucleic acid according to claim 33 which encodes a peptide of SEQ ID NO 4, 6, 7, 29, 21, 22, 23, 24, 25, 26, 27, 28 or the linker peptide shown in Figure 34.

25 35. A nucleic acid according to claim 33 which encodes a peptide comprising SEQ ID NO 77 linked at the C-terminal end of SEQ ID NO 4, 6, 7, 29, 21, 22, 23, 24, 25, 26, 27, 28 or the linker peptide shown in Figure 34